Sequence of the superoxide dismutase 1 (SOD 1) gene in familial Parkinson’s disease

O Bandmann, M B Davis, C D Marsden, A E Harding

Abstract

Mutations in the superoxide dismutase 1 (SOD 1) gene have been detected in affected members of some families with familial amyotrophic lateral sclerosis. To evaluate the possibility of a shared genetic defect in amyotrophic lateral sclerosis and Parkinson’s disease, the SOD 1 gene was sequenced in index patients with familial Parkinson’s disease from 23 families. No changes were detected.

(Keys: Parkinson’s disease; amyotrophic lateral sclerosis; superoxide dismutase)

Oxidative stress, resulting from increased free radical production or defects in antioxidant systems, seems to play an important part in the pathogenesis of neurodegenerative diseases such as amyotrophic lateral sclerosis and Parkinson’s disease. Oxidative stress in the substantia nigra in Parkinson’s disease is indicated by increased concentrations of lipid hydroperoxides and decreased content of reduced glutathione. The increased concentration of iron and decreased concentration of ferritin in the substantia nigra might enhance free radical production. Enzymes such as superoxide dismutase (SOD) and glutathione peroxidase in brain normally prevent or limit tissue damage from oxidation derived free radicals. SOD 1 is a Cu/Zn metalloenzyme that produces hydrogen peroxide by the dismutation of superoxide, a free radical. Rosen and colleagues showed that some patients with familial amyotrophic lateral sclerosis have mutations of the SOD 1 gene. Mutations of SOD 1 have since been detected in 13% to 20% of kindreds with familial amyotrophic lateral sclerosis; they are heterogeneous and occur in four of the five exons. Intracellular SOD exists as two isoenzymes with different subcellular localisations. The major form, the copper/zinc-dependent SOD 1, occurs largely in the cytosol, whereas the minor form, the manganese-dependent SOD 2, is found mainly in the mitochondrial matrix. Data on activity of SOD 1 and SOD 2 in the substantia nigra of patients with Parkinson’s disease are conflicting. Some authors have found an increase in cytosolic, but not particulate, SOD activity, whereas others reported an increased activity of the particulate fraction with normal activity of the cytosolic form. Some, but not all, mutations of the SOD 1 gene found in familial amyotrophic lateral sclerosis cause reduced enzyme activity.

Amyotrophic lateral sclerosis and Parkinson’s disease may occur in the same patient more often than would be expected by chance. There is also some evidence of nigrostriatal damage in amyotrophic lateral sclerosis. Positron emission tomography of patients with amyotrophic lateral sclerosis and no clinical signs of parkinsonism has shown a progressive fall in 6-fluorodopa uptake, and consistently reduced uptake in patients with amyotrophic lateral sclerosis of long duration. Cell loss in the substantia nigra has been observed in at least studies of amyotrophic lateral sclerosis brains.

A shared genetic susceptibility for both amyotrophic lateral sclerosis and Parkinson’s disease has been suggested. Amyotrophic lateral sclerosis and Parkinson’s disease are clinically distinct, but mutations in different functional domains of SOD 1 could result in phenotypic variation. Because transgenic mice with increased SOD 1 activity show resistance to the neurotoxin MPTP, abnormal SOD 1 activity in Parkinson’s disease could result in increased susceptibility of dopaminergic neurons to neurotoxins such as MPTP.

Familial Parkinson’s disease is not easily amenable to linkage analysis due to small family size and the possibility of genetic heterogeneity. To circumvent these problems, we have sequenced the SOD 1 gene in 23 patients with familial Parkinson’s disease to evaluate the possibility of a shared genetic defect in both familial amyotrophic lateral sclerosis and familial Parkinson’s disease. Although a brief report suggested that mutations of this gene are not detected in sporadic cases of Parkinson’s disease, they are also rare in sporadic amyotrophic lateral sclerosis. Exclusion of the SOD 1 region by linkage analysis in three families with Parkinson’s disease would not exclude a pathogenic role of SOD 1 mutations if, as is likely, there is substantial genetic heterogeneity as found in familial amyotrophic lateral sclerosis.
Patients and methods

Patients

Blood samples were obtained from members of 23 British and Irish families, selected on the basis of the index case having clinically typical Parkinson’s disease and at least one affected living relative. Nineteen of these families were reported previously, as were diagnostic criteria. The SOD1 coding region was entirely sequenced in one affected member of each family.

Methods

DNA was extracted from blood by standard techniques. The five exons of the SOD1 gene were amplified with the polymerase chain reaction with the following primer pairs: 5’ TAA AGT AGT CGC GGA GAC GGG and 5’ CGG CCT CGC AAC ACA AGC CT (exon 1), 5’ GCA GTT AGG CAG CTT GGT GG and CCC ACC TGC TGC TGT ATT ATC TCC (exon 2), 5’ AAA TAG GCT GTA CCA CGA CTG and 5’ AGT GTT TGA CAG TAC TGC TC (exon 3), 5’ CAT ATA GGC ATG TTG GAG ACT and 5’ AGA GTT TAT CTG GAT CTG AG (exon 4), GTA TTG TTG GGA GGA GGT AGT GAT and 5’ TTC TAC AGC TAG CAG GAT AAT (exon 5). Conditions for the polymerase chain reactions were: exon 1: one cycle at 92°C for three minutes, followed by 32 cycles of 92°C for one minute, 62°C for three minutes, and 72°C for two minutes; exons 2–5: one cycle at 92°C for three minutes, followed by 30 cycles of 92°C for 30 seconds, 58°C (exon 2), 57°C (exon 3), 45°C (exon 4), or 62°C (exon 5) for 30 seconds, and one cycle of 72°C for 30 seconds, followed by a final extension time of 10 minutes at 72°C. Products of the polymerase chain reaction were purified with Magicprep (Promega) and rendered single stranded by capture on to streptavidin coated beads (Dynal). Direct sequencing was performed with fluorescent labelled dye terminators and an automated sequencer (Applied Biosystems). The internal primers used for sequencing were: 5’ TTG CCT CGT CAG TCC TGG CAA (exon 1), 5’ TTC AGC AAC TCT CTC CAA CTT TGC AC (exon 2), 5’ CAT ATG AGT CAG CAA GTT CA (exon 3), 5’ CGC GAC TAA CCA TCA AAG TG (exon 4), and 5’ CAG TTT CTC ACT ACA GGT AC (exon 5). Sequences were compared with the published sequence (Genbank accession number: X01662, X01781–4).

Results

No changes were detected in the sequence of the SOD1 gene in any of the 23 index patients with familial Parkinson’s disease.

Discussion

There is increasing evidence that there may be a genetic component in the pathogenesis of Parkinson’s disease. There is an increased incidence of Parkinson’s disease among the relatives of patients with Parkinson’s disease, and large pedigrees apparently exhibiting autosomal dominant inheritance of Parkinson’s disease have been described. Allelic association has been reported for the debrisoquine hydroxylase CYP2D6 locus, but this gene does not account for familial aggregation of Parkinson’s disease. Our results show that changes in the coding region of the SOD1 gene, a reasonable candidate gene for familial Parkinson’s disease, are unlikely to contribute to the pathogenesis of this disease.

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